UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/359,592	07/23/99	GARVER	JHV-009.01 R

HM12/1226

025181
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EXAMINER	
NGUYEN, Q	
ART UNIT	**PAPER NUMBER**
1632	10

DATE MAILED: 12/26/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

1- File Copy

PTO-90C (Rev. 2/95)

Office Action Summary	Application No.	Applicant(s)
	09/359,593	GARVER ET AL.
	Examiner	Art Unit
	Quang Nguyen	1632

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 October 2000*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,2 and 4-49* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,2 and 4-49* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. & 119(e).

Attachment(s)

15)☒ Notice of References Cited (PTO-892)

16)☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

17)☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s). *8*

18)☐ Interview Summary (PTO-413) Paper No(s). _____

19)☐ Notice of Informal Patent Application (PTO-152)

20)☐ Other: .

DETAILED ACTION

Applicant's amendment filed October 16, 2000 in Paper no. 9 is acknowledged. Claims 1-2, 4-49 are pending in the present application.

Response to Amendments

The rejection of claims 29, 32 and 40 under 35 U.S.C. § 112, second paragraph is withdrawn in view of Applicant's amendments. The rejection of claims 37 and 42 is maintained for reasons stated below.

The rejection of claims 1-15, 21, 23-28, 36, 37, 39-47 under 35 U.S.C. § 102 (a) is maintained because the Patent Office has not received the Declaration under 37 C.F.R. 132.

The rejection of claims 1, 2, 29 and 48 under 35 U.S.C. § 102 is withdrawn in view of Applicant's amendments.

The rejection of claims 1-28, 30-31, 33, 35-37, 39-47 under 35 U.S.C. § 103 is withdrawn in view of Applicant's amendments.

Upon careful review of the present application, the following is a new ground of rejection and responses to Applicant's arguments.

Specification

A substitute specification including the claims is required pursuant to 37 CFR 1.125(a) because the top spacing margin of the submitted specification is not proper, and missing texts occur due to hole punching.

A substitute specification filed under 37 CFR 1.125(a) must only contain subject matter from the original specification and any previously entered amendment under 37 CFR 1.121. If the substitute specification contains additional subject matter not of record, the substitute specification must be filed under 37 CFR 1.125(b) and must be accompanied by: 1) a statement that the substitute specification contains no new matter; and 2) a marked-up copy showing the amendments to be made via the substitute specification relative to the specification at the time the substitute specification is filed.

Double Patenting

Claim 25 is objected to under 37 CFR 1.75 as being a substantial duplicate of claim 26. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k).

Claim 25 is directed to a composition of claim 24, wherein said viral vector contains a transgene. A transgene is a nucleic acid encoding a recombinant gene product, and as such claim 25 is essentially the same invention as that recited in claim 26.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 29-34, 38, and 48 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The factors to be considered in the determination of an enabling disclosure have been summarized as the quantity of experimentation necessary, the amount of direction or guidance presented, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art and the breadth of the claims. *Ex parte Forman*, (230 USPQ 546 (Bd Pat. Appl & Unt, 1986); *In re Wands*, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988)).

Claim 29 is directed to a gene delivery system for transducing cells of a host comprising a coacervate microsphere encapsulating at least a nucleic acid and a delivery agent for facilitating intracellular delivery of said nucleic acid, wherein upon administration of said coacervate microsphere to a host, a controlled release of said nucleic acid results in transduction of cells of said host by said nucleic acid. Claims 30-34 are drawn to a method for delivering a nucleic acid, preferably one encodes a therapeutic agent, to a host comprising administering to a host a composition

comprising a coacervate disclosed by the instant claimed invention, wherein said administration of said composition results in controlled release of the transfer vector, preferably a viral vector, *in vivo*. Claim 38 is directed to a method for the sustained release of a virus to a target site comprising providing to the targeting site a coacervate microsphere comprising a coacervate of gelatin and alginate having a virus incorporated therein. Claim 48 is drawn to a gene delivery system for transfecting a cell with a viral vector encapsulated and released from the coacervate of the instant invention.

The specification teaches the preparation of microspheres made by the coacervation of gelatin and alginate in the presence of recombinant adenovirus containing a luciferase expression cassette. It further revealed that the variation in the microsphere composition and the cross-linking modulates the amount and released pattern of recombinant virus in *in vitro* assays. Lyophilization of adenovirus within the microspheres was also shown to minimize the bioactive loss in comparison to the lyophilization of free adenovirus. With a human lung cancer engrafted on nude mouse model, it was demonstrated that bioactive adenovirus were released *in vivo* from the microspheres that were injected intratumorally, as evident by the luciferase activity in harvested tumor nodules. The above evidence is noted and considered, however, the evidence can not be extrapolated to the instantly claimed invention which when read in light of the specification is drawn to methods of delivering a nucleic acid to a host or the sustained release of a virus to a target site presumably in a host and a gene delivery system for transducing cells of a host or for transfecting a cell with a viral vector for the purposes of gene therapy and nucleic acid immunization (See page 3 of the

specification, lines 1-5 and 17-19). As enablement requires the specification to teach how to make and **use** the claimed invention, the instant specification fails to enable the **use** of methods for delivering a nucleic acid to a host and a gene delivery system for gene therapy and genetic immunization.

Regarding to the gene therapy aspect of the claims, the specification is not enabled for the claimed invention because it fails to provide guidance or direction for one skilled in the art to use the claimed methods of delivery a nucleic acid to a host or the gene delivery system to obtain any therapeutic effect as contemplated by Applicant for a plethora of diseases, disorders or genetic defects such as Duchenne and Becker muscular dystrophy, adenosine deaminase deficiency, cancer, Parkinson's, Alzheimer's, AIDS among many others (specification, pages 39-41). There is no specific guidance as to promoters, vectors or dosages that are utilized to treat a particular disease, disorder or a genetic defect. Moreover, there is no correlation between the luciferase activity detected in harvested tumor nodules that had been treated with coacervate microspheres containing recombinant adenoviruses of this invention with the desired therapeutic results expected for the treatment of aforementioned diseases, disorders and genetic defects. As the art does not teach such a correlation nor provide such guidance, it is incumbent upon the specification to do so. Additionally, at the effective filing date of the present application, gene therapy was still considered to be immature and highly unpredictable. Given the lack of guidance or direction provided by the instant specification, it would have required undue

experimentation without a predictable expectation of success for one skilled in the art to make and **use** the claimed invention.

As noted in the previous Office Action mailed on 07/06/2000 in Paper No. 7 that there are several factors limiting an effective gene therapy, and these include sub-optimal vectors, the lack of stable gene expression, and most importantly an efficient gene delivery to target cells or tissues. The specification fails to provide teachings showing that a gene construct in the coacervate microsphere of the instant invention could provide an efficient therapeutic transgene expression in targeted cells or tissues that results in desirable treatment outcomes for any and all diseases contemplated by the present application. Wivel and Wilson (cited in the previous Office Action) noted that an efficient gene therapy vector has not existed, and regarding the failure of the instant specification to provide guidance or direction for a skilled artisan how to make and use an efficient gene therapy vector other than those already known in the art, it would have required undue experimentation without a predictable expectation of success to use the claimed invention.

The claims also encompass the utilization of a nucleic acid encoding any and all therapeutic agents to be incorporated in the coacervate microspheres to treat aforementioned diseases, disorders and genetic defects. However, the specification fails to address issues such as the fate of delivering recombinant gene transfer vectors, the fraction of vectors taken up by targeted cells once they are released from coacervate microspheres, the level of mRNA produced, the stability of the recombinant protein produced, the recombinant protein's compartmentalization and its bioactive

activity. These factors differ dramatically based on which recombinant protein being produced to treat which disease or disorder, and the desired therapeutic effect being sought. Therefore, the level of gene expression, its duration and its *in vivo* therapeutic effects are not always predictable, and hence they can not be overcome by routine experimentation. With the lack of guidance and direction provided by the specification, it would have required undue experimentation without a predictable expectation of success for a skilled artisan to make and **use** the instant invention.

Regarding to the deliverance of a transgene encoding a therapeutic agent to a target cell or tissue of a host via coacervate microspheres, the specification fails to provide sufficient guidance or teachings on vector targeting to specific tissues or cells in the subject. At the effective filing, vector targeting *in vivo* to desired tissues, organs continues to be unpredictable and inefficient. This is supported by numerous teachings available in the art. For example, Miller & Vile (FASEB 9:190-199, 1995) reviewed the types of vectors available for *in vivo* gene therapy, and concluded that ""for the long-term success as well as the widespread applicability of human gene therapy, there will have to be advances .... Targeting strategies outlined in this review, which are currently only at the experimental level, will have to be translated into components of safe and highly efficient delivery systems" (page 198, column 1). Deonarain (Exp. Opin. Ther. Patents 8:53-69, 1998) indicated that one of the biggest problems hampering successful gene therapy is the "ability to target a gene to a significant population of cells and express it at adequate levels for a long enough period of time" (page 53, first paragraph). Deonarain also reviewed new techniques under experimentation in the art

which show promise, but is currently even less efficient than viral gene delivery (see page 65, first paragraph under Conclusion section). Verma & Somia (Nature 389:239-242,1997) reviewed various vectors known in the art for use in gene therapy and the problems which are associated with each and clearly indicated that at about the time of the claimed invention resolution to vector targeting had not been achieved in the art (see the entire article). Verma & Somia also discussed the role of the immune system in inhibiting the efficient targeting of viral vectors such that efficient expression is not achieved (see page 239, and second and third columns of page 242). Verma & Somia also indicated that appropriate enhancer-promoter sequences can improve expression, but that the "search for such combinations is a case of trial and error for a given cell type" (page 240, sentence bridging columns 2 and 3). The specification fails to provide sufficient guidance for a skilled artisan to overcome the unpredictability of vector targeting, such that efficient gene transfer and expression is achieved in specific target tissues or cells via coacervate microspheres in order to attain desired therapeutic results.

With regard to the nucleic acid immunization aspect of the instant claims, the state of the art is new and unpredictable at the effective filing date of the present application. Chattergoon et al. (FASEB J. 11:753-763, 1997) stated that "Though DNA vaccines have shown promise in animal models and have raised hopes, the technology is considered an emerging technology" (column 1, paragraph 2, page 762) and "There is little evidence that the immune response induced by these vaccines will be completely protective against any human pathogen" (page 762, paragraph bridging

columns 1-2). Most recently, Leitner et al. (Vaccine 18:765-777, 2000) further stated that "Although genetic vaccines have been significantly improved, they may not be sufficiently immunogenic for therapeutic vaccination of patients with infectious disease or cancer in clinical trials" (Abstract, page 765). Leitner et al. also listed several variable factors affecting the immunogenicity of genetic vaccines. These include: the structure of the plasmid backbone, amount of plasmid delivered, expression levels of the antigen, age and strain of the particular species, target tissue, and route of immunization among others (See Table 1, page 767). It is also recognized that the animal model should correlate to the disease conditions studied. Furthermore, it is impossible to predict whether an untested antigen of an infectious pathogen will elicit a protective immune response in a given type of animal and the route of administration was recognized as being a critical parameter determining whether protective immunity is elicited. Since the instant claims encompass any and all hosts, one skilled in the art has also recognized that results observed in animal model system following testing of a DNA expression vector-based agent are not predictive of outcome or efficacy in applications in other species of animal or in humans, due to differences in anatomy, cell biology, genetics, and immunology between different types of animals and between the animal models and humans. This is further supported by the teachings of McCluskie et al. (Mol. Med. 5:287-300, 1999) who stated that "it is probably safe to say that any vaccine that works in a human will work in a mouse, but not necessarily vice versa. Therefore, it is difficult to predict from mouse studies the potential of a new vaccine for humans. In fact, in those human trials that have carried out, none of the DNA vaccines induced the strong

immune responses that had been seen in mice with the same vectors." (column 2, last paragraph, page 296). Against this background, the instant specification fails to provide any guidance or direction demonstrating that the claimed methods of delivering a nucleic acid to a host or the gene delivery system via coacervate microphores are effective for nucleic acid immunization purposes in any and all host for any and all diseases.

Additionally, the physiological art is recognized as unpredictable (MPEP 2164.03). As set forth in *In re Fisher*, 166 USPQ 18 (CCPA 1970), compliance with 35 USC 112, first paragraph requires:

> That scope of claims must bear a reasonable correlation to scope of enablement provided by specification to persons of ordinary skill in the are; in cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws; in cases involving unpredictable factors, such as most chemical reactions and physiological activity, scope of enablement varies inversely with degree of unpredictablity of factors involved.

Accordingly, due to the lack of guidance and direction provided by the specification, the unpredictability and current state of the gene therapy and nucleic acid immunization and physiological arts, and the breadth of the claims, it would have required undue experimentation for one skilled in the art without a predictable expectation of success to make and **use** the instantly claimed invention.

Claims 17-20, 22, 35, 39-47 rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the composition of a controlled release of a nucleic acid of the instant invention, a method for preparing the same, a

coacervate microsphere for transfection and expression of a recombinant protein *in vitro*, and a kit comprising microspheres and instructions for using said microspheres, does not reasonably provide enablement for other embodiments of the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Claims 17-20 and 22 are directed to the composition for controlled release of a nucleic acid for claim 15, wherein the administration of the microsphere to a patient results in controlled release of the expression vector, and wherein the delivery agent facilitates intracellular delivery of the expression vector producing a recombinant protein, preferably an antigen in the patient. Claim 35 is drawn to a kit containing a gene delivery system comprising microspheres and instructions for using said microspheres, wherein said microspheres are comprised of a cationic molecule and an anionic molecule and said microspheres encapsulate a virus. Claim 39 is directed to a method for preparing a pharmaceutical preparation, comprising combining a pharmaceutically acceptable excipient with a coacervate of cationic and anionic molecules wherein a recombinant virus is encapsulated in said coacervate. Claims 40-46 are directed to a method for preparing a gene delivery system, while claim 47 is drawn to a coacervate microsphere for transfection and expression of a recombinant protein prepared by a process of the instant invention, wherein release of the virus from the coacervate and transfection of cells by the virus *in vivo* or *in vitro* results in expresssion of said recombinant protein.

As noted above, enablement requires the specification to teach how to make and **use** the claimed invention, the instant specification fails to enable the **use** of the coacervate microsphere composition, a kit of the gene delivery system and methods of preparing the gene delivery system and a pharmaceutical composition comprising the coacervate microspheres for the same reasons set forth in the previous Office Action (pages 5-7) and more clearly stated in the rejection of claims 29-34, 38, and 48 above.

In responding to the previous Office Action, regarding to claim rejection under 112 U.S.C. § 112, first paragraph, Applicant argued that "The examiner has not provided any evidence in support of a position that the claimed coacervates would be ineffective if the expression cassette included, for example, a nucleic acid that has been therapeutically beneficial when administered by another vehicle." Applicants cited the works of Anderson & Blaese and Hung et al. referenced in the review of Dang et al. as examples of therapeutically beneficial nucleic acids and treatment regiments. The Examiner respectfully disagrees with the Applicant's assertion that the cited examples have demonstrated therapeutically beneficial nucleic acids and treatment regiments by these statements "Although the transduction of the ADA gene into the lymphocytes of patients afflicted by immunodeficiency by Anderson and Blaese was accomplished, the data indicate that **no therapeutic effect has been gained** from this initial study." (page 471, column 2, lines 1-3); "The result suggests that E1A-liposome gene therapy is feasible, and **additional clinical trials on patients with less-malignant cancer are required to evaluate therapeutic efficacy**." (page 474, column 1, last sentence of the

first full paragraph). Clearly, therapeutic effects have not been established in the cited examples. With regarding to the abstracts of Kawamura et al. and Ariga et al., they are not found in the application and therefore the Examiner can not determine whether they support the Applicant's argument or not. With regard to the cited reference of Wivel and Wilson in the previous Office Action, Applicant argued that "It is sufficient for patentablility if the claimed compositions and methods are capable of inducing a desirable therapeutic effect. As stated above, a number of gene therapy techniques have shown efficacy, and a number of clinical trials are currently underway testing the safety and efficacy of gene therapy." As noted above, the examples that Applicant cited have not shown any therapeutic effects as asserted by the Applicant. Applicant also argued that the suboptimal longterm gene expression means that repeated doses may be required, and that this limitation does not represent an obstacle to patentability. The Examiner would like to point out that unlike nude mouse models, repeated applications of coacervate microspheres releasing recombinant viral vectors will induce adverse host immune responses against delivered vectors, that in effect inhibiting effective vector targeting and transgene expression in desired cells or tissues, and preventing desired therapeutic effects to be achieved. Verma & Somia noted the role of the immune system in inhibiting the efficient targeting of viral vectors such that efficient expression is not achieved (see page 239, and second and third columns of page 242). With respect to the Eck and Wilson reference cited in the previous Office Action, Applicant argued that Eck and Wilson provide successful *in vivo* uses of gene therapy as indicated by Table 5-1 listing a large number of approved clinical trials. The Examiner finds the

argument is unpersuasive because Table 5-1 does not indicate that therapeutic effects have been achieved for any gene therapy protocol. The abstract of Klatzmann et al. has not been provided in the application, and therefore the Examiner can not evaluate whether therapeutic effects have been achieved in that study through gene therapy.

In summary, the Examiner finds Applicant's arguments to be unpersuasive. In addition, due to the lack of guidance and direction provided by the specification, the unpredictability and current state of the gene therapy and nucleic acid immunization and physiological arts, and the breadth of the claims for reasons set forth above, the claim rejection under 112 U.S.C. § 112, first paragraph, is maintained.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 8, 9, 17-20, 22, 24, 37, 40, 42, 44 and 47 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 8 and its dependent claim 9 recite the limitation "said crosslinking agent" in the claims. There is insufficient antecedent basis for this limitation in the claim. Claims 8 and 9 are dependent on claim 2 which does not recite crosslinking agent. Appropriate correction is required.

Claims 17 and its dependent claims recite the limitation "administration" in line 1 of the claim. There is insufficient antecedent basis for this limitation in the claim. Claim 15 from which claim 17 is dependent upon is a composition claim. Claims 17-20 and 22

are confusing because they are composition claims, yet they contain a method step, for example administration. Clarification is needed and appropriate correction is requested.

In claim 24, the phrase "said nucleic acid is a viral vector, and said delivery agent is a virus of said viral vector" is unclear because a virus or a recombinant virus is normally considered to be a viral vector. Clarification is needed because the metes and bounds of the claim can not be clearly determined. Similarly in claim 31, the phrase "said transfer vector is a viral vector, said delivery agent is a virus of said vector, and said viral vector is enveloped in said virus" is also unclear for the reason stated above.

In claim 37, the phrase "recombinant virus or a natural virus which has been engineered to be used as a vector" is unclear. What is the difference between the two? Since the claim language is not clear, the metes and bounds of the claim can not be clearly established.

The term "substantially" in claims 40 and 44 is a relative term which renders the claim indefinite. The term "substantially" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. Which percentages of coacervates which are microspheres would be considered to be substantial, 80%, 90%, 95% or 99%? Or how aqueous a solution is to be considered "substantially aqueous"?

In claim 42, the phrase "said nucleic acid comprises a viral vector, said delivery agent comprises a virus particle corresponding to said viral vector, and said viral vector

is encapsulated in said virus particle" is unclear and wordy. A recombinant virus is a viral vector having all stated components.

Claim 47 is unclear and indefinite because although it is a composition claim, yet it contains method steps recited as "release of said virus from said coacervate and transfection of cells by said virus *in vivo* or *in vitro* results in expression of said recombinant protein" in the last two lines of the claim. Appropriate correction is requested.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

Claims 1,2, 4, 5, 7, 11, 13, 14 and 15 are rejected under 35 U.S.C. 102(e) as being anticipated by Truong et al. (U.S. Patent No. 6,025,337).

The claims are drawn to a composition for controlled release of a nucleic acid comprising (a) a coacervate, (b) a nucleic acid incorporated in said coacervate, and (c) a delivery agent incorporated in said coacervate, wherein the coacervate comprises a cationic molecule and an anionic molecule other than said nucleic acid; the same composition wherein said coacervate is a microsphere, wherein said nucleic acid is a transfer vector having a transgene, wherein said cationic molecule is a gelatin, wherein

said transfer vector comprises at least one regulatory element, preferable a promoter, and wherein said transfer vector comprises an expression cassette.

Truong et al. disclosed a solid microparticle or microsphere (column 7, line 8) for delivering of nucleic acids to and transfection of target cells comprising gelatin (cationic molecule), nucleic acid, chondroitin sulfate (anionic molecule), wherein a molecular species (delivering agent) is attached to the surface of the microparticle, wherein the molecular species is selected from the group consisting of a targeting ligand and a linking molecule (See claims 1 and 15, column 3, lines 38-41). Truong et al. also taught that micorspheres can be synthesized by the coacervation of gelatin and chondroitin sulfate (column 1, lines 64-65). Truong et al. disclosed the incorporated nucleic acid in the microsphere is the LAMP-a cDNA, a plasmid cDNA with a mouse LAMP-1 gene inserted into an Invitrogen plasmid cDNA with a CMV promoter (column 6, lines 11-14). The reference further taught that glutaraldehyde, carbodiimides can be used for crosslinking of linking molecules to promote stability of the microparticle or microsphere (column 4, lines 4-14). The disclosed microparticle comprises from 5% to 30% (w/w) nucleic acids (Claim 12), and is less than 3 μm in size.

Therefore, the reference clearly anticipates the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the

invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1, 2, 4, 6, 16, 23-28 and 35 are rejected under 35 U.S.C. 103(a) as being unpatentable over Truong et al. (U.S. Patent No. 6,025,337) in view of Beer et al. (Adv. Drug Delivery Reviews 27:59-66, 1997).

The claims are drawn to a composition for controlled release of a nucleic acid comprising (a) a coacervate, (b) a nucleic acid incorporated in said coacervate, and (c) a delivery agent incorporated in said coacervate, wherein the coacervate comprises a cationic molecule and an anionic molecule other than said nucleic acid; the same composition wherein said compostion is a microsphere, and wherein said nucleic acid is a viral vector, and said delivery agent is a virus; the same wherein said viral vector contains a transgene, preferably the gene product is an antigen, and wherein the virus is selected from the group consisting of recombinant retrovirus, adenovirus, adeno-associated virus and herpees simplex-virus-1. Claim 35 is directed to a kit containing a gene delivery system, comprising microspheres and instructions for using said

microspheres, wherein said microspheres are comprised of a cationic molecule and an anionic molecule and said microspheres encapsulate a virus. Regarding to claim 35, the intended use of the kit is not given any patentable weight in art rejection.

Truong et al. disclosed a solid microparticle or microsphere (column 7, line 8) for delivering of nucleic acids to and transfection of target cells comprising gelatin (cationic molecule), nucleic acid, chondroitin sulfate (anionic molecule), wherein a molecular species (delivering agent) is attached to the surface of the microparticle, wherein the molecular species is selected from the group consisting of a targeting ligand and a linking molecule (See claims 1 and 15, column 3, lines 38-41). Truong et al. also taught that microspheres can be synthesized by the coacervation of gelatin and chondroitin sulfate (column 1, lines 64-65). Truong et al. disclosed the incorporated nucleic acid in the microsphere is the LAMP-a cDNA, a plasmid cDNA with a mouse LAMP-1 gene inserted into an Invitrogen plasmid cDNA with a CMV promoter (column 6, lines 11-14). The reference further taught that glutaraldehyde, carbodiimides can be used for crosslinking of linking molecules to promote stability of the microparticle or microsphere (column 4, lines 4-14). The disclosed microparticle comprises from 5% to 30% (w/w) nucleic acids (Claim 12), and is less than 3 μm in size. However, Truong et al. did not teach specifically the incorporation of a recombinant virus into the disclosed microparticle or microsphere or a kit comprising the same.

Beer et al. disclosed a composition of poly (lactic-glycolic) acid (PLGA) microspheres containing a recombinant adenovirus, AdRSVntlacZ. After injection into the striatum of mice with microspheres containing AdRSVntlacZ, beta-galactosidase

activity was detected in harvested brains after 7 days, and a dose dependent increase in beta-galactosidase activity was also noted (see Fig. 4). Although viable virus could be delivered both *in vitro* and *in vivo* from the PLGA microspheres, optimal microencapsulation yield, virus stability, and efficient transfer remained elusive (second column, second paragraph, page 63). Beer et al. suggested that different polymers should be investigated for their ability to allow for sustained release of recombinant viral vectors (column 2, last paragraph, page 63). It should be noted that beta-galactosidase is also an antigen upon administering into a host.

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time of invention was made to modify a microsphere composition disclosed by Truong et al. by replacing a plasmid vector with a recombinant adenovirus taught by Beer et al. to arrive at the instant claimed invention. One of ordinary skilled in the art would have been motivated to carry out such modification to improve the microencasulation yield and virus stability once encapsulated for the poly (lactic-glycolic) acid (PLGA) microspheres taught by Beer et al. in order to improve the effectiveness of adenoviral-mediated gene transfer for glioma therapy (Beer et al., page 60, column 2, second full paragraph and page 63, column 2, first full paragraph). Beer et al. even suggested that other methods and different polymers should be investigated for their ability to allow sustained release of recombinant viral vectors as noted above. One of ordinary skilled in the art would have selected the microparticle delivery system taught by Truong et al. for its advantages over the system used by Beer et al. Truong et al. stated that "Compared to other synthetic polymeric systems, such as the extensively

studied polylactic/polyglycolic copolymers, the mild conditions of microparticle formulation are appealing. Unlike the solvent evaporation and hot-melt techniques used to formulate synthetic polymeric microparticles, complex coacervation requires neither contact with organic solvents nor heat. It is also particularly suitable for encapsulating bio-macromolecules such as nucleic acids not only through passive solvent capturing but also by direct charge-charge interactions." (column 5, lines 1-10). Due to the relative milder conditions for the preparation of coacervation microparticles taught by Truong et al., one would reasonably expect that recombinant retrovirus should be encapsulated and still retains its activity, because it does in the system described by Beer et al. Moreover, regarding to the relatively large size of adenovirus (approximately 100 nm), the size of the microspheres taught by Truong et al. (less than 3 μm) is large enough to encapsulate large viral particles. Moreover, it has been shown that decreased sphere size results in improvement in encapsulation yield (Beer et al., page 63, lines 1-3 of the first full paragraph). A kit comprising the modified microspheres resulting from the combined teachings of Truong et al. and Beer et al. would have also been obvious.

Thus, the claimed invention as a whole was *prima facie* obvious in the absence to the contrary.

Claims 1 and 49 are rejected under 35 U.S.C. 103(a) as being unpatentable over Truong et al. (U.S. Patent No. 6,025,337) in view of Casey et al. (Oncogene 6:1791-1797, 1991).

The claim is drawn to a composition of claim 1, wherein the nucleic acid encodes a polypeptide which inhibit cell proliferation.

The teachings of Truong et al. have been discussed above. However, Truong et al. did not teach an incorporation of a plasmid vector containing a DNA sequence encoding a polypeptide that inhibits cell proliferation. Casey et al. disclosed an expression plasmid vector comprising a wild type DNA sequence of p53 whose expression inhibits cell growth for human breast cancer MDA-MB 468 and T47D cell lines having mutated copies of p53 (See abstract and page 1793, column 2, last sentence continues to column 1 on page 1794). Therefore, the claimed invention would have been obvious since one of ordinary skilled in the art would have replaced the plasmid vector disclosed by Truong et al. with one taught by Casey et al. for inhibiting certain cancer cell populations having mutated p53 gene. Thus, the claimed invention as a whole was *prima facie* obvious in the absence to the contrary.

Claims 40-47 are rejected under 35 U.S.C. 103(a) as being unpatentable over Leong et al. (U.S. Patent No. 5,759,582, PTO-1449 # 6, AB) in view of Truong et al. (U.S. Patent No. 6,025,337) and Beer et al. (Adv. Drug Delivery Reviews 27:59-66, 1997).

The claims are drawn to a method for preparing a gene delivery system in which the microspheres prepared from the coarcevation of a cationic molecule and an anionic molecule encapsulate a nucleic acid, preferably a recombinant virus, and a coacervate microsphere for transfection and expression of a recombinant protein prepared from the

same method. It should be noted that the intended use of the coacervate and the gene delivery system is not given any patentable weight in art rejection.

Leong et al. (US Patent No. 5,759,582) taught a method for preparing a pharmaceutical composition in the form of microspheres, comprising the following steps: (a) providing a gelatin (a cationic molecule) aqueous solution; (b) providing a chondroitin sulfate (an anionic molecule) aqueous solution; (c) adding a therapeutically effective amount of a pharmaceutically active substance either to the solution in step (a) or to the solution in step (b); (d) mixing the gelatin and chondroitin sulfate solutions to form a coacervate suspension; (e) adding a crosslinking agent to the coacervate suspension to crosslink the coacervates, the coacervates encapsulating the pharmaceutically active substance; and (f) incubating the coacervate suspension to form microspheres and recovering the microspheres. (column 2 in summary of invention). Leong et al. further taught that after recovering the microspheres, they may be washed and dried in a standard techniques, e.g., lyophilization (column 4, last paragraph). However, Leong et al. did not teach a process of preparing a coacervate microsphere which encapsulates a nucleic acid.

Truong et al. disclosed a solid microparticle or microsphere (column 7, line 8) for delivering of nucleic acids to and transfection of target cells comprising gelatin (cationic molecule), nucleic acid, chondroitin sulfate (anionic molecule), wherein a molecular species (delivering agent) is attached to the surface of the microparticle, wherein the molecular species is selected from the group consisting of a targeting ligand and a linking molecule (See claims 1 and 15, column 3, lines 38-41). Truong et al. also taught

that microspheres can be synthesized by the coacervation of gelatin and chondroitin sulfate (column 1, lines 64-65). Truong et al. disclosed the incorporated nucleic acid in the microsphere is the LAMP-a cDNA, a plasmid cDNA with a mouse LAMP-1 gene inserted into an Invitrogen plasmid cDNA with a CMV promoter (column 6, lines 11-14). The reference further taught that glutaraldehyde, carbodiimides can be used for crosslinking of linking molecules to promote stability of the microparticle or microsphere (column 4, lines 4-14). The disclosed microparticle comprises from 5% to 30% (w/w) nucleic acids (Claim 12), and is less than 3 μm in size. However, Truong et al. did not teach specifically the incorporation of a recombinant virus into the disclosed microparticle or microsphere.

Beer et al. disclosed a composition of poly (lactic-glycolic) acid (PLGA) microspheres containing a recombinant adenovirus, AdRSVntlacZ. After injection into the striatum of mice with microspheres containing AdRSVntlacZ, beta-galactosidase activity was detected in harvested brains after 7 days, and a dose dependent increase in beta-galactosidase activity was also noted (see Fig. 4). Although viable virus could be delivered both *in vitro* and *in vivo* from the PLGA microspheres, optimal microencapsulation yield, virus stability, and efficient transfer remained elusive (second column, second paragraph, page 63). Beer et al. suggested that different polymers should be investigated for their ability to allow for sustained release of recombinant viral vectors (column 2, last paragraph, page 63).

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time of invention was made to modify a method of preparing a coarcevate

microsphere disclosed by Leong et al. (US Patent No. 5,759,582) with the combined teachings of Truong et al. and Beer et al., by substituting a pharmaceutical composition comprising water soluble protein, peptide, glycoproein, or mixture thereof in step (c) with a recombinant adenovirus to arrive at the instantly claimed invention, including a coacervate microsphere prepared by the same process. The motivations for one of ordinary skilled artisan to carry out the above modification are provided by both Truong et al. and Beer et al. One of ordinary skilled in the art would have been motivated to carry out such modification to improve the microencasulation yield and virus stability once encapsulated for the poly (lactic-glycolic) acid (PLGA) microspheres taught by Beer et al. in order to improve the effectiveness of adenoviral-mediated gene transfer for glioma therapy (Beer et al., page 60, column 2, second full paragraph and page 63, column 2, first full paragraph). Beer et al. even suggested that other methods and different polymers should be investigated for their ability to allow sustained release of recombinant viral vectors as noted above. One of ordinary skilled in the art would have selected the microparticle delivery system taught by Truong et al. for its advantages over the system used by Beer et al. Truong et al. stated that "Compared to other synthetic polymeric systems, such as the extensively studied polylactic/polyglycolic copolymers, the mild conditions of microparticle formulation are appealing. Unlike the solvent evaporation and hot-melt techniques used to formulate synthetic polymeric microparticles, complex coacervation requires neither contact with organic solvents nor heat. It is also particularly suitable for encapsulating bio-macromolecules such as nucleic acids not only through passive solvent capturing but also by direct charge-

charge interactions." (column 5, lines 1-10). Due to the relative milder conditions for the preparation of coacervation microparticles taught by Truong et al., one would reasonably expect that recombinant retrovirus should be encapsulated and still retains its activity, because it does in the system described by Beer et al. Moreover, regarding to the relatively large size of adenovirus (approximately 100 nm), the size of the microspheres taught by Truong et al. (less than 3 μm) is large enough to encapsulate large viral particles. Moreover, it has been shown that decreased sphere size results in improvement in encapsulation yield (Beer et al., page 63, lines 1-3 of the first full paragraph).

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claims 1, 2, 4, 8, 9, 10, 12, 21, 36 and 37 are rejected under 35 U.S.C. 103(a) as being unpatentable over Leong et al. (U.S. Patent No. 5,759,582, PTO-1449, paper no. 6, AB) in view of Truong et al. (U.S. Patent No. 6,025,337) and Beer et al. (Adv. Drug Delivery Reviews 27:59-66, 1997) as applied to claims 40-47 above, and further in view of Watts et al. (WO 98/30207, PTO-1449, paper no. 8, AL).

The claims are drawn to a composition for controlled release of a nucleic acid comprising (a) a coacervate, (b) a nucleic acid incorporated in said coacervate, and (c) a delivery agent incorporated in said coacervate, wherein the coacervate comprises a cationic molecule and an anionic molecule other than said nucleic acid; the same composition wherein said coacervate is a microsphere, wherein said nucleic acid is a

transfer vector and the crosslinking agent for said microsphere is a metal cation comprises calcium, wherein said anionic molecule is alginate, wherein the cationic molecule is gelatin and the anionic molecule is alginate, and wherein the microsphere is lyophilized. Claims 36 and 37 are directed to a coacervate microsphere for sustained release of a virus comprising a coacervate of gelatin and alginate having a recombinant virus incorporated therein.

The combined teachings of Leong et al., Truong et al. and Beer et al. have been discussed above, and they do not suggest the formation of a coacervate microsphere wherein the anionic molecule is alginate and the cationic molecule is gelatin, and wherein the microsphere is crosslinked by a calcium metal ion. However, apart from chitosan-gelatin microparticles Watts et al. taught that the production of microspheres by complexation between a negatively charged material such as alginate and a positively charged chitosan (essentially coacervation) in the presence of calcium ion, usually for crosslinking purpose, has been described in the literature (page 4, last paragraph).

Accordingly, it would have been obvious to a person of ordinary skill in the art at the time of invention was made to further modify the coacervate microspheres resulted from the combined teachings of Leong et al., Truong et al. and Beer et al. for substituting chondroitin sulfate for alginate, and using calcium as a crosslinking agent to arrive at the instant claimed invention. The motivations for such a modification are already discussed. It is noted that Beer et al. suggested **different polymers should be**

investigated for their ability to allow sustained release of recombinant viral vectors for improving the effectiveness of adenoviral-mediated gene transfer for glioma therapy.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

In response to the previous Office Action, regarding to the references of Leong et al. (U.S. Patent No. 5,759,582, PTO-1449, paper no. 6, AB), Leong et al. (J. Controlled Rel. 53:183-193, 1998), Roy et al. (U.S. Patent No. 5,972,707) and Beer et al. (Adv. Drug Delivery Reviews 27:59-66, 1997), Applicant argued that "the Examiner has provided no teachings which would suggest 1) that coacervate nanospheres can be formed around viral particles; 2) that viral particles incorporated in coacervate nanospheres would retain their activity and facilitate intracellular delivery of nucleic acids". Applicant further argued that the nanospheres taught by Leong et al. or Roy et al. are orders of magnitude smaller (200-750 nm), possible too small to encapsulate large viral particles efficiently. Applicant argued that Leong et al. espouse the use of non-viral delivery techniques for ease of synthesis, cell tissue targeting, low immune response, and unrestricted plasmid size, and thus teach against this combination. The examiner respectfully find Applicant's arguments to be unpersuasive for the following reasons. Applicant has not provided any evidence or scientific reasons indicating that coacervate nanospheres disclosed by Leong et al., Roy et al. or microspheres of Truong et al. would not formed around viral particles. Viral particles can be encapsulated and they still retain the biological activity to mediate gene transfer under

relatively much harsher conditions during the preparation of poly (lactic-glycolic) acid (PLGA) microspheres taught by Beer et al. Therefore, one would not expect that viral particles would not be encapsulated or be inactivated by the coacervation process taught by Leong et al., Roy et al. and Truong et al. The sizes of the nanospheres (200-750 nm) and microspheres of Truong et al. (less than 3 μm) are large enough to encapsulate viral particles of approaximately 100 nm. To further improve the encapsulation yield of virus, one of ordinary skill in the art would prefer to encapsulate viral particles in microspheres of smaller size than those taught by Beer et al. (10-20 μm) because it has been shown that decreased sphere size results in improvements in encapsulation yield (Beer et al., page 63, lines 1-3, second full paragraph). Finally, although Leong et al., Roy et al. and Truong et al. prefer the incorporation of non-viral vectors in their coacervate microspheres, but by no means that they exclude the incorportation of viral vectors in their disclosed compositions. In certain situations, for example in glioma therapy, the use of recombinant adenoviruses in glioma gene therapy is preferred because they are easily engineered to express the genes desired for direct cell killing, for induction of host immune responses to tumor cells, or both, and they do not require mitotic activity for gene transfer or expression. At the effective filing date of the present application, there is a need to improve the effectiveness of adenoviral-mediated gene transfer for glioma therapy, and on the basis of the teachings provided by Leong et al., Roy et al., Truong et al. and Beer et al., the encapsulation of recombinant adenovirus in coacervate microspheres can improve the sustained release of low dose adenovirus to increase their applicability for glioblastoma treatment.

Conclusions

Claims 17-20, 22, 29-34, 38, 39 and 48 are free of prior art. At the effective filing date, the prior art does not teach or fairly suggest methods for delivering a nucleic acid to a host or a gene delivery system for transducing cells of a host using the coacervate microspheres disclosed by the instantly claimed invention.

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (703) 308-8339.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's mentor, Deborah Crouch, Ph.D., may be reached at (703) 308-1126, or SPE, Karen Hauda, at (703) 305-6608.

Any inquiry of a general nature or relating to the status of this application should be directed to Patent Analyst, Patsy Zimmerman, whose telephone number is (703) 305-2758.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1632.

Papers related to this application may be submitted to Group 160 by facsimile transmission. Papers should be faxed to Group 160 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15,1989). The CM1 Fax Center number is or (703) 305-3014 or (703) 308-4242.

Quang Nguyen, Ph.D.
Examiner, AU 1632

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